Molecular diversity and phytochemical variability in the Iranian poppy (Papaver bracteatum Lindl.): A baseline for conservation and utilization in future breeding programmes

Qaderi, Ardashir

2019-04

Qaderi , A , Omidi , M , Pour-Aboughadareh , A , Poczai , P , Shaghaghi , J , MehrAfarin , A , Nohooji , M G & Etminan , A 2019 , ' Molecular diversity and phytochemical variability in the Iranian poppy (Papaver bracteatum Lindl.): A baseline for conservation and utilization in future breeding programmes ' , Industrial Crops and Products , vol. 130 , pp. 237-247 . https://doi.org/10.1016/j.indcr

http://hdl.handle.net/10138/323866
https://doi.org/10.1016/j.indcrop.2018.12.079

Industrial Crops & Products 130 (2019) 237–247

Industrial Crops & Products

Molecular diversity and phytochemical variability in the Iranian poppy (Papaver bracteatum Lindl.): A baseline for conservation and utilization in future breeding programmes

Ardeshir Qaderi[a], Mansour Omidi[b], Alireza Pour-Aboughadareh[b], Peter Poczai[c], Javad Shaghaghi[d], Ali Mehrafarin[a], Majid Ghorbani Nohooji[a], Alireza Etminan[e,]

[a] Medicinal Plant Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran
[b] Department of Agronomy and Plant Breeding, Tehran University, Karaj, Iran
[c] Botany Unit, Finnish Museum of Natural History, University of Helsinki, P.O. Box 7, Helsinki FI-00014, Finland
[d] Department of Agronomy and Crop Breeding, Shahed University, Tehran, Iran
[e] Department of Plant Breeding, Kermanshah Branch, Islamic Azad University, Kermanshah, Iran

ARTICLE INFO

Keywords:
Poppy
Germplasm
Genetic diversity
Gene-targeted markers
Population structure
Thebaine
Morphine

ABSTRACT

In the present investigation, 72 accessions of the Iranian poppy (Papaver bracteatum Lindl.) were analyzed for genetic diversity and population structure using start codon targeted polymorphism (SCoT) and inter simple sequence repeat (ISSR) markers along with four important phytochemical traits to provide baseline knowledge for the Iranian poppy's breeding and conservation plans. Twelve ISSR and thirteen SCoT primers generated a total of 98 and 186 fragments with a mean of 8.17 and 14.31 fragments per primer, respectively. Polymorphic information content for ISSR and SCoT primers ranged from 0.39 to 0.45 and 0.28 to 0.34, with the resolving power ranging from 21.61 to 3.97 and 13.08 to 28.02, respectively. Neighbour-joining (NJ) based clustering grouped 72 accessions into three main groups based on two markers studied (ISSR and SCoT) and the combined data (ISSR + SCoT), which associated with their eco-geographical regions. Population structure based analysis divided 72 accessions into 3 subpopulations using ISSR markers, when SCoT was used eight subpopulations were observed. However, when the combined data was used only three subpopulations were found, which corre-sponded to the grouping observed with the NJ method and these results were supported by principal coordinate analyses (PCoA). Phytochemical analysis revealed that plant capsule has higher total amounts of the alkaloids; thebaine, morphine and oripavine than stem tissues. Interestingly, for the geographical parameters, latitude showed a significant and positive correlation with thebaine extracted from both stem and capsules and the regression results confirmed these associations. Taken together, our results indicated that three populations Ploor, Eil-Teymoor and Anjomane due to their high contents of alkaloids like thebaine as well as the Taham population due to its high content of morphine and oripavine have a strong enough potency to be used in the pharmacy industry.

1. Introduction

To date more than 200,000 natural products have been identified (Fiehn, 2002) and among them some alkaloids such as codeine, mor-phine and paclitaxel have been utilized as drugs (Newman and Cragg, 2012). Benzylisoquinoline alkaloids (BIAs) are widespread plant sec-ondary metabolites with approximately 2500 known members (Hagel et al., 2007). Morphine alkaloids are an important class of pharma-ceutical substances because of their influential analgesic (Niknam et al., 2010), antitussive and narcotic antagonist characteristics (Kyslikova et al., 2013). Opium poppy (Papaver somniferum L.), as a sole and illegal commercial resource of morphine which has low secondary metabolites accumulation (Nakagawa et al., 2011; Nyman, 1978). The Iranian or Persian poppy (P. bracteatum Lindl.) was described by Sharghi and Lalezari (1967) as a highly rich source of thebaine, commercially converted to codeine and semi synthetic opiates (Craker and Simon, 1991). Papaver bracteatum is a diploid species (2n = 14), which belongs to section Oxytona Bernh. in the core 'clade 2' of Papaver sensu stricto (Carolan et al., 2006) and has outcrossing pollination with gameto-phytic self-incompatibility (Goldblatt, 1974; Milo et al., 1998).

Corresponding author.
E-mail address: alietminan55@yahoo.com (A. Etminan).

https://doi.org/10.1016/j.indcrop.2018.12.079
Received 15 October 2018; Received in revised form 26 November 2018; Accepted 25 December 2018

Table 1
Inter-simple Sequence Repeats (ISSR) and Start Codon Targeted (SCoT) primers and their amplification results generated in the 72 P. bracteatum accessions.

Primer	Sequence	Ta (°C)	TAB	NPB	PPB	PIC	Rp	MI
ISSR1	GGAGAGGAGAGGAGA	44.9	8	8	100	0.40	11.5	3.23
ISSR2	AGAGAGAGAGAGAGAGYT[a]	46.2	10	10	100	0.39	14.55	3.96
ISSR3	ACACACACACACACACG	50	6	6	100	0.41	8.55	2.45
ISSR4	GAGAGAGAGAGAGAGAYC	45.5	5	5	100	0.44	6.66	2.22
ISSR5	GAGAGAGAGAGAGAGAA	42.7	8	8	100	0.42	11.08	3.40
ISSR7	TCGTCGTCGTCGTCGG	54.3	3	3	100	0.44	3.97	1.34
ISSR8	TCGTCGTCGTCGTCGC	54.9	5	5	100	0.43	6.88	2.14
ISSR9	CACACACACACACACART[a]	48.8	4	4	100	0.40	5.77	1.60
ISSR10	CTCCTCCTCCTCRC[†]	45.7	5	5	100	0.45	6.55	2.25
ISSR11	GAGAGAGAGAGAGAGAT	42.4	13	13	100	0.43	18.11	5.49
ISSR12	ACACACACACACACACC	49.8	15	15	100	0.43	20.63	6.44
ISSR13	ACACACACACACACACYG[a]	51.3	16	16	100	0.44	21.61	7.02
		Mean	8.17	8.17	100	0.42	11.32	3.46
SCoT1	CAACAATGGCTACCACCC	40	17	17	100	0.33	26.83	5.65
SCoT2	CAACAATGGCTACCACCG	40	14	14	100	0.31	22.50	4.41
SCoT3	CAACAATGGCTACCACCT	40	12	12	100	0.28	19.97	3.35
SCoT4	CAACAATGGCTACCACGA	40	17	17	100	0.29	27.83	5.04
SCoT6	CAACAATGGCTACCACGT	40	14	14	100	0.33	22.05	4.68
SCoT7	CAACAATGGCTACCAGCA	40	16	16	100	0.32	25.58	5.12
SCoT8	CAACAATGGCTACCAGCC	40	15	15	100	0.33	23.72	4.96
SCoT10	ACGACATGGCGACCAACG	40	18	18	100	0.34	28.02	6.20
SCoT11	ACGACATGGCGACCATCG	40	16	16	100	0.29	26.33	4.66
SCoT12	CCATGGCTACCACCGGCC	40	17	17	100	0.31	27.44	5.29
SCoT13	CACCATGGCTACCACCAT	40	8	8	100	0.29	13.08	2.38
SCoT14	CAATGGCTACCATTAGCC	40	12	12	100	0.32	19.11	3.89
SCoT15	CCATGGCTACCACCGCCA	40	10	10	100	0.29	16.38	2.95
		Mean	14.31	14.31	100	0.31	22.99	4.51

Ta temperature annealing, TAB total amplified bands, NPB number of polymorphic, PPB percentage of polymorphism, PIC polymorphism information content, Rp resolving power, MI marker index.

[a] Y = C or T and R = A or G.

Although P. bracteatum is naturally distributed in high altitudes from 1500 to 2500 m, the main distribution areas for this species have been found in the following three distinct areas: the Alborz mountains in the north of Iran, the Zagros Mountains in west and north western Iran and on the northern slope of the Caucasus in north western Iran and north eastern Turkey (Goldblatt, 1974).

Knowledge about the genetic diversity between plant species offers scenarios for germplasm conservation programmes and improving the plant characteristics (Kalia et al., 2011). One of the valuable tools for assessing genetic diversity are DNA-based molecular markers. Various molecular marker techniques with set parameters are currently avail-able for the study of genetic diversity and structure analysis. Previously, molecular systems such as RAPD (Randomly Amplified Polymorphic DNA; Williams et al., 1990), AFLP (Amplified Fragment Length Poly-morphism; Vos et al., 1995) and ISSR (Inter Simple Sequence Repeats; Godwin et al., 1997) that are collectively known as arbitrarily amplified DNA markers (AAD markers) (Wolfe and Liston, 1998) have been used in genetic diversity, population analysis studies, QTL mapping and di-agnostic genomic fingerprinting (Tiwari et al., 2016). A comprehensive review of molecular markers with detailed information about them was done by Poczai et al. (2013). Among various molecular marker tech-nique, inter simple sequence repeats (ISSRs) have been widely used for assessment of genetic diversity and population structure studies of various plant species (Li and Jin, 2008; Moradkhani et al., 2015; Tiwari et al., 2016; Etminan et al., 2016; Pour-Aboughadareh et al., 2017; Etminan et al., 2018b). ISSR are DNA fragments which located between adjacent, oppositely oriented microsatellite regions. These primers due to their longer sequence and higher annealing temperature generate reliable and reproducible bands (Zietkiewicz et al., 1994). Furthermore, the cost of the analyses of this technique is lower than other molecular techniques such as SSR, RFLP and AFLPs (Wang et al., 2008). In the past decade, progress in the molecular tools has led to the use of gene-tar-geted markers and the development novel DNA-based marker tech-nique. Start codon-targeted (SCoT) polymorphism is one of the novel, simple, and reliable gene-targeted marker technique, which has been developed based on the short conserved region of the translation in-itiation codon (ATG) on both DNA strands (Collard and Mackill, 2009). Due to its various advantages like high polymorphism, rateability and low cost, this technique has been successfully applied in genetic studies of many plant species (Bhattacharyya et al., 2013; Rajesh et al., 2015; Feng et al., 2015; Etminan et al., 2016, 2018a; Pour-Aboughadareh et al., 2017, 2018).

Since, the genetic characteristics of the Iranian poppy germplasm were not investigated before, we aimed to assess the genetic diversity and population structure of 72 P. bracteatum accessions using SCoT and ISSR markers. Comparison of different populations based on phyto-chemical traits was another goal of this study. We believe that the re-sults obtained in this study will be useful in opening up new prospects for the conservation and utilization of the germplasm of Iranian poppy.

2. Materials and methods

2.1. Plant materials and DNA extraction

The experimental materials included seventy-two accessions of the Iranian poppy (P. bracteatum) collected from different eco-geographical regions of Iran. Detailed geographical information of these materials is given in Supplementary Table S1. Seeds of each accession were planted in Murashige and Skoog medium (MS) with 1:4 concentration of macro and micro elements. Plantlets were grown in the optimal growth con-dition in phytotron (16 h light and 8 h dark, 23 ± 2 °C). Fresh leaves from each accession were sampled for DNA extraction. The total genomic DNA was isolated according to the CTAB protocol (Doyle and Doyle, 1987) with minor modifications. DNA quality was checked by 0.8% agarose gel electrophoresis.

2.2. Genotyping with ISSR markers

For ISSR analysis, 12 primers were selected based on literatures (Table 1). All PCR amplifications were carried out in a 30 μl reaction

mixture containing 4 μl of the temple DNA, 1.5 μl of each primer, 15 μl of master mix 2XPCR (ready-to-use PCR master mix 2X; Ampliqon) and 9.5 μl double distilled water. ISSR-PCR amplification was done under the following conditions: initial denaturation at 95 ℃ for 10 min, fol-lowed by 38 cycles of denaturation at 95 °C for 1 min, annealing at 42–54 °C (varied for each primer) for 50 s, extension at 72 °C for 2 min and a final extension at 72 °C for 7 min. PCR products were visualized on a 1.5% agarose gel, stained with SafeView II and finally photo-graphed using a gel documentation system. Moreover, a 100 Kb DNA ladder was used as the standard.

2.3. Genotyping with SCoT markers

Thirteen SCoT primers (Table 1) were designed based on Collard and Mackill (2009). All the tested primers amplified scorable poly-morphic bands. PCR reactions were performed in a 30 μl reaction as described previously. PCR reactions were amplified under the following conditions: initial denaturation at 95 °C for 10 min followed by 38 cy-cles of denaturation at 95 °C for 1 min, annealing at 45 °C (for all pri-mers) for 45 s and extension at 72 °C for 2 min followed by final ex-tension at 72 °C for 7 min. PCR products separation and visualization was done as described for genotyping with ISSR markers.

2.4. Phytochemical assay

In order to phytochemical assay, the amount of total alkaloids content of thebaine, morphine and oripavine in both plant stem and capsules were evaluated. For this purpose, 10 capsules and related stems from each population sampled in their natural habits. Capsules and stems were transferred to room temperature, and then the dried materials were ground to powder. 50 mg of obtained powders were subjected to methanol extraction (75%) in an ultrasonic bath at 40 °C. The extracts were resolved by reversed phase High-performance liquid chromatography (AZURA preparative HPLC system, KNAUER, Germany). Chromatographic assay was done by use of C-18 column
(150 × 2.00 mm, particle size 5 μm) and terms of running setting were: injection volume: 10 μl, flow rate: 0.2 ml/min, mobile phase: 0.1 N NH3: 0.1 N NH4Cl with a pH of 8.8: 100% acetonitrile, detection wa-velength: 280 nm). The content of total alkaloid, morphine, thebaine and oripaveine were estimated based on the special calibration curve of each alkaloid (Dittbrenner et al., 2008). Finally, alkaloid components were recorded based on percent on dry matter (PDM).

2.5. Data collection and analysis

All observed clear polymorphic bands in the ISSR and SCoT profiles were scored as 1 or 0 on the basis of the presence and absence of the bands, respectively. The discriminatory powers of the used primers were assessed through informativeness parameters; including total amplified bands (NPB), number of polymorphic bands (PPB), poly-morphism Information Content (PIC), resolving power (Rp), and the marker Index (MI). Analysis of molecular variance (AMOVA) was done with purpose of partitioning of genetic variation between and within populations. Also, distribution of accessions was assessed with Principal Coordinate Analysis (PCoA). These analyses were computed using GenAlEX ver. 6.5 software (Peakall and Smouse, 2006). Seven genetic variation parameters including the observed (Na) and effective (Ne) numbers of alleles, Nei's gene diversity (H), Shannon's information index (I), the percentage of polymorphic loci (PPL), inter-population differentiation (G_{ST}), and gene flow (Nm) were estimated using POP-GENE ver. 1.31 software (Yeh et al., 1997). Genetic dissimilarities were estimated based on Jaccard's similarity coefficient (Jaccard, 1908) using DARwin ver. 6 software (Perrier et al., 2003). Neighbour-joining dendrograms were rendered to show the relationships between dif-ferent accessions. Structure analysis was done using STRUCTURE soft-ware version 2.3.4 (Pritchard et al., 2000) to show the Bayesian clustering patterns for the 72 studied accessions. A consecutive series of K were done from 2 to 9 in 5 independent runs with the initial burn-in period was set to 50,000 followed by 50,000 Markov Chain Monte Carlo (MCMC) iterations. A program available online, STRUCTURE HARVE-STR (Earl and Vonholdt, 2012) was used to calculate the K.

Data obtained by HPLC detection were analyzed based on com-pletely randomized block design with six replications. Means compar-ison of alkaloids was done by Duncan's Multiple Range Test (DMRT) at
$P < 0.05$ for different populations. Also Differences among the means of stem and capsules' alkaloid and it's components were determined using the t-test. A Principal component analysis (PCA) was used to study the interrelationships between different phytochemical traits and geographical data. To obtain raw geographical data we used the online program Convert Geographic Units (available at http://www.rcn.montana.edu/resources/converter.aspx) and converted geographical data to quantitative data. Other quantitative analyses like comparison means and regression analysis were performed using the XLSTAT package (Addisonsoft XLSTAT, 2008).

3. Results

3.1. ISSR and SCoT polymorphism

In this study, 25 ISSR and SCoT primers were used to evaluate the genetic polymorphism of P. bracteatum. A summary of the informa-tiveness parameters estimated for ISSR and SCoT primers is shown in Table 1. All the chosen ISSR primers amplified a total of 98 amplified fragments across the 72 accessions, and they were all polymorphic. The number of polymorphic fragments ranged from 3 (ISSR7) to 16 (ISSR13) with an average of 8.17. The polymorphism information content (PIC) varied between 0.39 and 0.45 with a mean of 0.42. The maximum and minimum values of PIC were recorded for ISSR2 and ISSR10, respectively. The average of resolving power (Rp) was 11.32 and ISSR13 showed the highest value (21.61), while the lowest (3.97) Rp belonged to ISSR7. The marker index (MI) parameter ranged from 1.34 (ISSR7) to 7.02 (ISSR13) with an average of 3.46. In the SCoT analysis, 13 primers generated 186 loci, all of which were polymorphic fragments. The number of polymorphic fragments ranged from a minimum of 8 in primer SCoT13 to a maximum of 18 in SCoT10. The PIC parameter varied between 0.28 and 0.34 with a mean of 0.31 per primer. The primers SCoT13 and SCoT10 showed the lowest and highest value for PIC, respectively. The average of Rp of 13 SCoT pri-mers was 22.99, and the highest value showed in primer SCoT10 (28.02). The MI parameter ranged from 2.89 (SCoT13) to 6.20 (SCoT10) with an average of 4.51.

3.2. Genetic diversity analysis

An analysis of molecular variance (AMOVA) was performed to de-tect differences between and within populations of P. bracteatum (Table 2). The results of AMOVA analysis indicated that percentage molecular variance was higher among populations (ISSR = 51%, SCoT = 55%, pooled data = 53%) than within populations (ISSR = 49%, SCoT = 45%, pooled data = 47%). The genetic differentiation coefficient (G_{ST})/gene flow (Nm) for ISSR, SCoT and pooled data were 0.51/0.48, 0.54/0.42 and 0.53/0.44, respectively. The population ge-netic diversity analysis using ISSR primers indicated the highest values of Shannon information (I = 0.23), Nei's gene diversity (He = 0.15) and percentage of polymorphic loci (PPL = 40.82%) among the Taham population, whereas the lowest values of these parameters (I = 0.11, He = 0.07, PPL = 22.45%) was in the Mamhimah population (Table 3). The observed (Na), effective (Ne) and private (PA) number of alleles ranged between 1.03–1.29, 1.12–1.27 and 0–3, respectively. The Taham and Mamhimah populations had the highest and lowest values of Na, Ne and PA, respectively. Furthermore, in SCoT/pooled data (ISSR + SCoT) analysis, the highest values of I (0.21/0.22), He (0.14/

Table 2
Analysis of molecular variance (AMOVA) in P. bracteatum populations.

Source of variation	ISSR		SCoT		ISSR + SCoT	
	Between Populations	Within Populations	Between Populations	Within Populations	Between Populations	Within Populations
df	5	66	5	66	5	66
SS	390.403	389.083	716.611	603.667	1107.014	992.750
MS	78.081	5.895	143.322	9.146	221.403	15.042
Est.Var	6.015	5.895	11.181	9.146	17.197	15.042
Var	51%	49%	55%	45%	53%	47%
PhiPT	0.505		0.550		0.533	
	P = 0.010					
G_{ST}	0.511		0.542		0.532	
Nm	0.478		0.421		0.439	

SS sum of squares, MS mean squares, Est. Var estimated variance components, Var total variance, Gst inter-population differentiation, Nm gene flow.

0.15), PPL (35.48%/37.32%), Na (1.26/1.27) and Ne (1.25/1.26) were found for the Taham population. In SCoT analysis, the lowest values of genetic parameters were detected among the Anjomane population, while in the pooled data analysis the lowest values of these parameters was recorded among the Mamhimah population. In the last analysis, the highest private number of alleles was detected among the Mamhimah population.

3.3. Genetic distances and groping accessions

The binary data from ISSR and SCoT primers subjected to estimate Jaccard distance coefficient pairs of accessions. In ISSR analysis, the pairwise genetic distance coefficients computed by Jaccard's coefficient indicated a range of 0.014–0.488 with an average value of 0.273 among all the 72 accessions of P. bracteatum. The maximum genetic distance (0.488) was observed between accessions Tah-3 from Taham and Eil-10 from the Eil-Teymoor population, while the minimum distance (0.014) was observed between accessions Mam-8 and Mam-9 both from the Mamhimah population (data not shown). Besides, the genetic distance coefficients by SCoT data varied between 0.013 and 0.396 with a mean value of 0.218. The maximum distance (0.396) was showed between accessions Tah-3 from Taham and Eil-7 from the Eil-Teymoor popula-tion, whereas the minimum distance (0.013) was showed between ac-cessions Eil-3 and Eil-4 both from the Eil-Teymoor population. The pairwise genetic distance coefficient computed using ISSR + SCoT data showed a range of 0.047–0.388 with a mean value of 0.236 among all the 72 studied accessions. The maximum distance was observed be-tween accessions Tah-1 from Taham and the Mam-3 population, while the minimum distance was obtained between accessions Anj-1 and Anj-2 both from the Anjomane population (data not shown).

The cluster analysis carried out by the neighbour-joining (NJ) method revealed clear interrelationships among the 72 accessions be-longing to 6 different populations. The dendrogram rendered by ISSR data clustered all accessions into three main groups (A–C). As shown in Fig. 1A, the first further divided into two subgroups. Eleven accessions from Eil-Teymoor (Eil 1–11) were included in subgroup AI, while subgroup AII including one accession from the Eil-Teymoor population (Eil-12) and all 24 accessions of the Anjomane (Anj 1–12) and Mam-himah (Mam 1–12) populations. The second main cluster also divided into two subgroups, so that all accessions of Taham comprised subgroup BI and all twelve accessions of the Ploor population placed in subgroup BII. Finally, all accessions of the Siah-Bisheh population generated a distinct cluster (C). The dendrogram generated by SCoT data indicated a clear clustering pattern among the studied accessions (Fig. 1B). All accessions clustered into three major groups (AeC) and each of them further divided into two subgroups. In cluster A, the first cluster separated all the accessions of the Anjomane and Eil-Teymoor populations into two distinct subgroups (AI and AII). The second cluster separately grouped all accessions of the Ploor (subgroup BI) and Siah-Bisheh (subgroup BII) populations into two distinct subgroups. Moreover, two

Table 3
Estimated genetic variation parameters for different Iranian poppy populations through ISSR and SCoT markers.

Marker	Population	PA	Na	Ne	I	He	PPL
ISSR	Ploor	0	1.24 ± 0.06	1.25 ± 0.04	0.20 ± 0.03	0.14 ± 0.02	35.71
	Siah-Bisheh	1	1.11 ± 0.06	1.16 ± 0.03	0.14 ± 0.02	0.09 ± 0.01	27.55
	Taham	3	1.29 ± 0.07	1.27 ± 0.04	0.23 ± 0.03	0.15 ± 0.02	40.82
	Eil-Teymoor	0	1.25 ± 0.07	1.23 ± 0.03	0.20 ± 0.02	0.13 ± 0.02	38.78
	Anjomane	0	1.10 ± 0.04	1.15 ± 0.03	0.14 ± 0.02	0.09 ± 0.01	27.55
	Mamhimah	0	1.03 ± 0.06	1.12 ± 0.02	0.11 ± 0.02	0.07 ± 0.01	22.45
	Mean		1.17 ± 0.02	1.2 ± 0.01	0.17 ± 0.01	0.11 ± 0.01	32.14 ± 2.99
SCoT	Ploor	0	1.18 ± 0.04	1.22 ± 0.03	0.17 ± 0.02	0.13 ± 0.02	29.03
	Siah-Bisheh	0	1.21 ± 0.04	1.21 ± 0.03	0.17 ± 0.02	0.12 ± 0.01	28.49
	Taham	0	1.26 ± 0.04	1.25 ± 0.03	0.21 ± 0.02	0.14 ± 0.02	35.48
	Eil-Teymoor	1	1.12 ± 0.04	1.18 ± 0.02	0.15 ± 0.02	0.10 ± 0.02	24.73
	Anjomane	0	1.08 ± 0.04	1.15 ± 0.02	0.12 ± 0.01	0.09 ± 0.01	20.43
	Mamhimah	4	1.09 ± 0.04	1.16 ± 0.02	0.13 ± 0.01	0.08 ± 0.01	20.97
	Mean		1.16 ± 0.02	1.19 ± 0.02	0.16 ± 0.01	0.11 ± 0.01	26.52 ± 2.32
ISSR + SCoT	Ploor	0	1.20 ± 0.04	1.23 ± 0.02	0.18 ± 0.01	0.13 ± 0.02	31.34
	Siah-Bisheh	1	1.17 ± 0.03	1.19 ± 0.02	0.16 ± 0.01	0.11 ± 0.01	28.17
	Taham	3	1.27 ± 0.04	1.26 ± 0.02	0.22 ± 0.01	0.15 ± 0.02	37.32
	Eil-Teymoor	1	1.17 ± 0.04	1.20 ± 0.02	0.16 ± 0.01	0.11 ± 0.02	29.58
	Anjomane	0	1.09 ± 0.03	1.15 ± 0.01	0.13 ± 0.01	0.09 ± 0.01	22.89
	Mam himah	4	1.07 ± 0.03	1.14 ± 0.01	0.12 ± 0.01	0.08 ± 0.01	21.48
	Mean		1.16 ± 0.01	1.19 ± 0.01	0.16 ± 0.01	0.11 ± 0.01	28.46 ± 2.37

PA private alleles, Na observed number of alleles, Ne effective number of alleles, I Shannon's information index, He Nei's gene diversity, PPL the percentage of polymorphism.

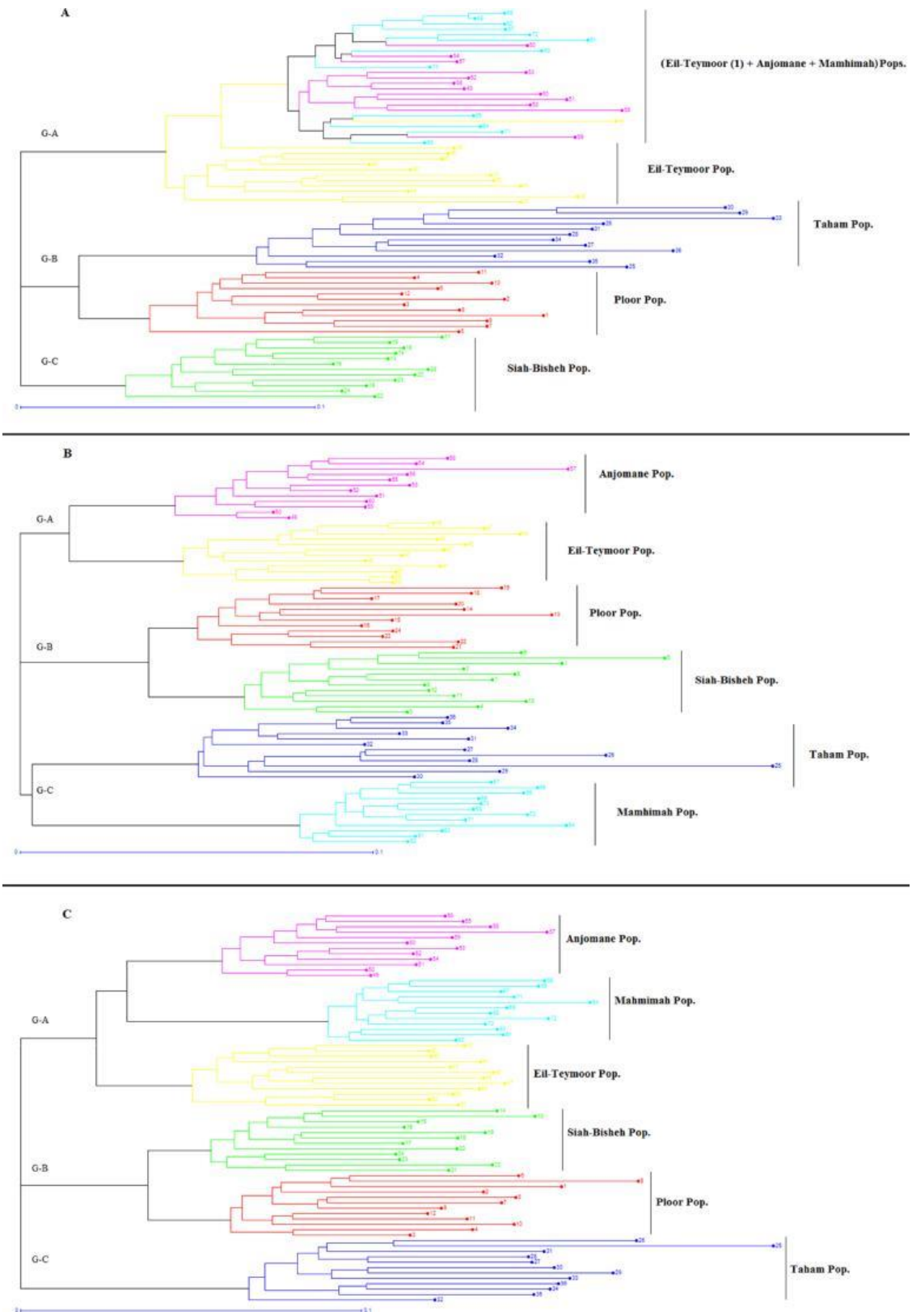

Fig. 1. Dendrogram of the 72 accessions of Iranian poppy (P. bracteatum) rendered by Jaccard's coefficients using neighbor-joining clustering method based on ISSR (A panel), SCoT (B panel) and the combined data (C panel).

populations Taham and Mamhimah separately clustered into two dif-ferent subgroups, subgroup CI and CII respectively, and together formed the third cluster (C). When the pooled data was subjected to cluster analysis, the dendrogram showed that all the accessions were clustered three major groups (AeC) (Fig. 1C). The first cluster divided into three subgroups (AI, AII and AIII) and each of them embraced all the accessions of Anjomane, Mamhimah and Eil-Teymoor populations, respectively. The second cluster separated populations of Siah-Bisheh

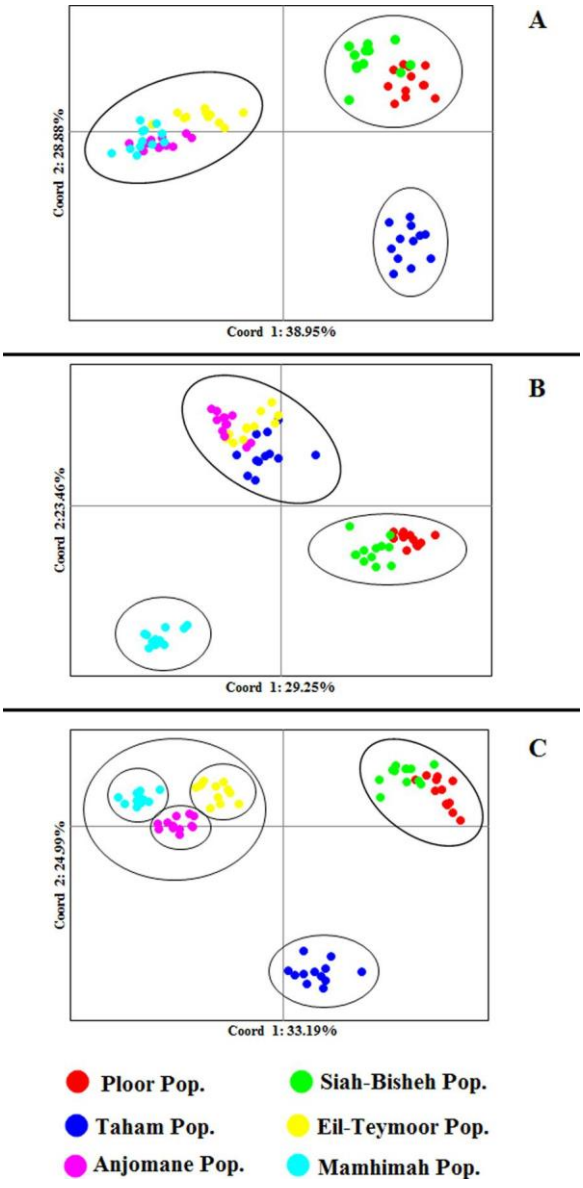

Fig. 2. Biplot derived from the PCoA of the 72 P. bracteatum accessions based on ISSR (A panel), SCoT (B panel) and the combined data (C panel).

(subgroup BI) and Ploor (subgroup BII) from each other and grouped them into two subgroups. All the accessions of the Taham population alone generated the third cluster (C).

3.4. Principal coordinate analyses (PCoA)

Principal coordinate analysis (PCoA) was performed based on the data of ISSR, SCoT and their combination. In ISSR based analysis the first two axes justified 67.83% (Coord1 = 38.95% and Coord2 = 28.88%) of the total variation and all the accessions from the Taham population were clustered into a distinct group (G1). All the Ploor and Siah-Bisheh accessions were clustered together in the same group (G2). Group 3 included all accessions from the Anjomane, Eil-Teymoor and Mamhimah populations (Fig. 2A). In PCoA for SCoT markers, the first two coordinates explained 52.71% of the total var-iation and accessions from the Anjomane, Eil-Teymoor and Mamhimah populations were tightly clustered into group G1. Similarly, all acces-sions belonging to the Ploor and Siah-Bisheh populations were placed in group G2, while the Mamhimah accessions created group G3 (Fig. 2B) far from other accessions. PCoA based on combined data indicated that the top two coordinates accounted for 58.18% of the total variation (Coord1 = 33.19% Coord2 = 24.99%). Although the last two PCoA analyses showed a clear grouping pattern for the 72 studied accessions, PCoA based combined data showed an eco-geographical isolation for different populations. As shown in Fig. 2C, group G1 included the Ploor and Siah-Bisheh accessions, group G2 with three distinct subgroups comprised the Eil-Teymoor, Mamhimah and Anjomane accessions and finally group G3 embraced all the Taham accessions as a separate group.

3.5. Population structure analysis

The population structure of the 72 P. bracteatum accessions was computed based on ISSR, SCoT and pooled data (ISSR + SCoT) with the Bayesian-based STRUCTURE package. Structure analysis identified three subpopulations (K = 3) with ISSR primers. Out of 72 accessions, subpopulation 1 (SP-1) contained all 36 accessions from Eil-Teymoor (12), Anjomane (12) and Mamhimah (12), while SP-2 contained 24 accessions from Ploor (12) and Siah-Bisheh (12) populations. Twelve accessions of Taham population were grouped into SP-3 (Fig. 3A). As shown in Fig. 3B, we identified two pike-points of K (K = 8 and K = 5) using SCoT data. The inferred subpopulation for K = 8 indicated that 72 accessions were placed into the following subpopulations–SP-1 (11): accessions from the Taham population; SP-2 (1): one accession from Taham; SP-3 (12): accessions from the Anjomane population; SP-4 (11): accessions from the Ploor populations; SP-5 (1): one accession from the Ploor population; SP-6 (12): accessions from the Mamhimah population; SP-7 (12): accessions from the Eil-Teymoor population; and SP-8 (12): accessions from the Siah-Bisheh population. At K = 5, the grouping pattern of accessions was clearer than at K = 8. In this pat-tern, all accessions from the Anjomane and Eil-Teymoor populations were assigned to SP-1, whereas all accessions from the Siah-Bisheh, Ploor, Mamhimah and Taham populations separately were grouped into the SP-2, SP-3, SP-4 and SP-5, respectively (Fig. 3B). Three distinct subpopulations were obtained with 25 polymorphic ISSR and SCoT primers (Fig. 3C). Twenty-four accessions belonging to the Ploor and Siah-Bisheh populations were assigned to SP-1. Twelve accessions from the Taham population and thirty-six accessions from the Eil-Teymoor, Anjomane and Mamhimah populations were assigned to SP-2 and SP-3, respectively.

3.6. Comparative analysis of the populations in terms of their phytochemical components

In order to compare different populations in terms of the most im-portant phytochemical components that exist in the stem and capsule organs of P. bractateum, a HPLC analysis was performed. As shown in Fig. 4, there was significant differences between plant stem and cap-sules in terms of oripavine, morphine, thebaine and total alkaloid content components. Oripavine was only observed in the Taham po-pulation (0.38 percent on dry matter) and this alkaloid was not ob-served in other populations. The morphine content ranged from 0.009 (in the stems) to 0.023 percent on dry matter (in the capsules) and the highest content in the stems was observed for Taham (0.04 percent on dry matter) followed by Anjomane (0.009 percent on dry matter) and Siah-Bisheh (0.007 percent on dry matter). In contrast, the Taham, Mamhimah and Siah-Bisheh populations by 0.023, 0.016 and 0.015 percent on dry matter, respectively showed the highest morphine content in their capsules. Thebaine, another important component, varied between 0.05 (in the stems) and 1.27 percent on dry matter (in the capsules), and the highest thebaine content in both stems and capsules was observed in the Eil-Teymoor and Ploor populations. The total alkaloid content showed a high range of variability among studied populations and ranged from 0.36 (in the stems) to 2.02 percent on dry matter (in the capsules). The highest total alkaloid content obtained from stem/capsules was found in the Mamhimah (1.27/2.02 percent on

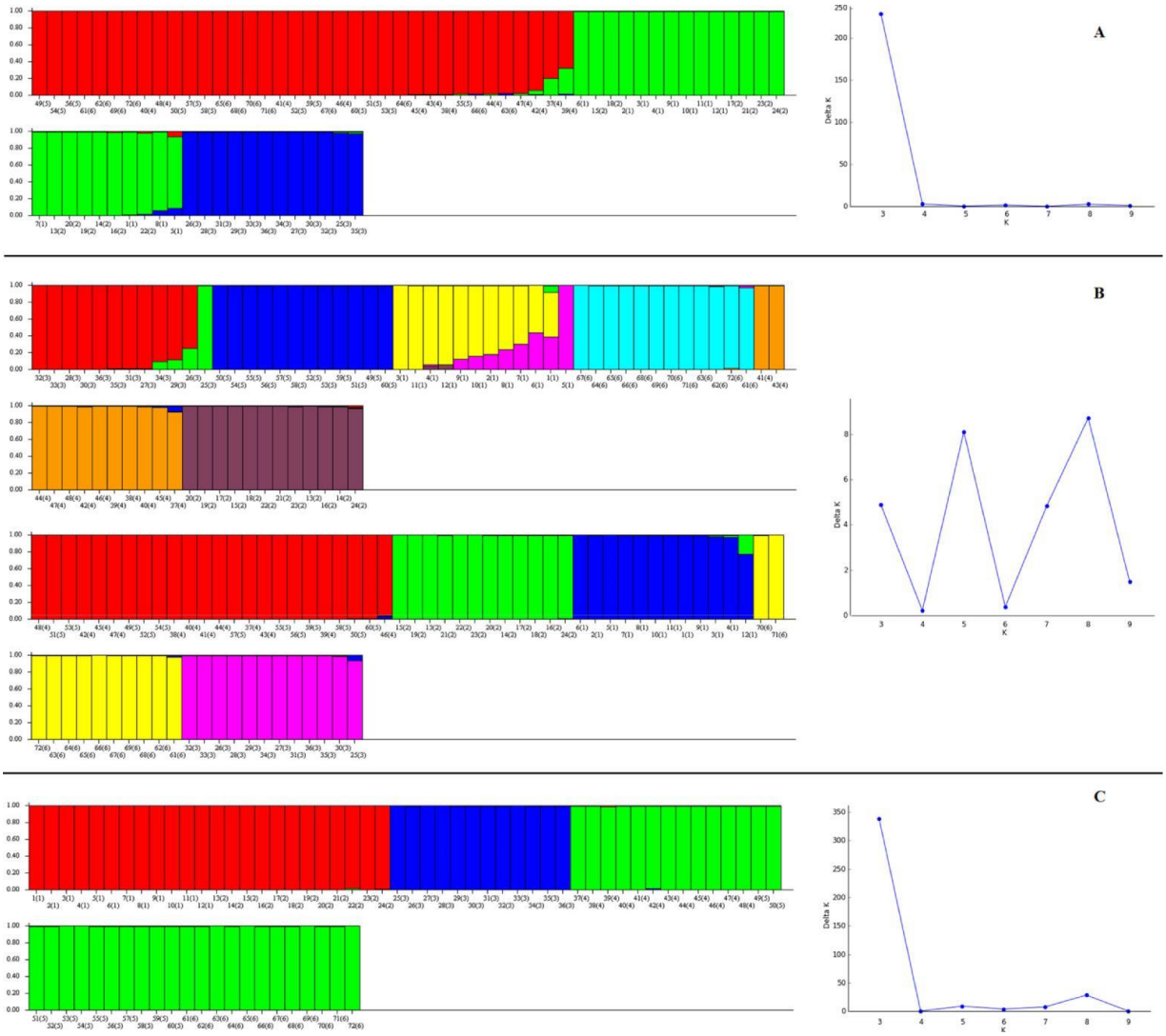

Fig. 3. Population structure of 72 P. bracteatum accessions using ISSR (A panel), SCoT (B panel) and the combined data (C panel).

dry matter) and Ploor (0.87/1.94 percent on dry matter) populations, while the lowest was found in the Taham population (0.36/1.20 per-cent on dry matter).

3.7. Relationships between geographical and phytochemical data

In order to study interrelationships in the geographical data—in-cluding longitude, latitude and elevation from the sea level—and phytochemical components a principal components analysis was com-puted. PCA results showed the top two first components (PCs) explained 88.72% of the total variation of phytochemical traits. PC1 accounted for 69.45% of the total variation and was positively influenced by thebaine and total alkaloid content for both stem and capsule, whereas PC2 justified 19.72% of the total variation and was mainly correlated with all phytochemical traits (except stem thebaine) and longitude. The rendered biplot based on PC1 and PC2 was used to explore the asso-ciation between phytochemical traits using the angles between the trait vectors on the biplot (Fig. 5). Thus small or large angles show a strong positive or weaker correlation, respectively. Also, a 180° angle indicates if there was a negative correlation among the traits, whereas a 90° angle would result if there was no correlation. The results showed morphine significantly correlated with oripavine content. The correlations be-tween thebaine and total alkaloid also were positive and significant. Of the various geographical parameters, only latitude showed a significant positive correlation for thebaine and total alkaloid contents. The result of linear regression analysis confirmed this result so that as shown in Fig. 5B, there is a positive relationship between thebaine and latitude, and two populations (Ploor and Eil-Teymoor) revealed superior the-baine content.

4. Discussion

The genetic diversity of Papaver bracteatum—which is known as the Iranian poppy and Persian poppy (Sharghi and Lalezari, 1967) —germplasm needs to be known for fast-tracking the development of breeding programs, as well as for the conservation and utilization of Iranian poppy germplasm resources. Molecular characterization using different types of marker systems (i.e., RAPD, AFLP, ISSR, SSR, SNP markers) has been showed to be an efficient and inexpensive system to estimate genetic diversity, and to determine the genetic structure of many plant species. During the past decade, several novel gene-based markers have been developed to aid studies of genetic diversity and population structure analyses. One of these gene-based marker techni-ques is start codon-targeted polymorphism (SCoT). This marker system has good capabilities in genetic studies because of its display poly-morphism in the conserved regions and its high reliability. Prior to this study, the Iranian poppy had not been studied because of its specific distribution and the difficulty of finding samples. Our findings may be

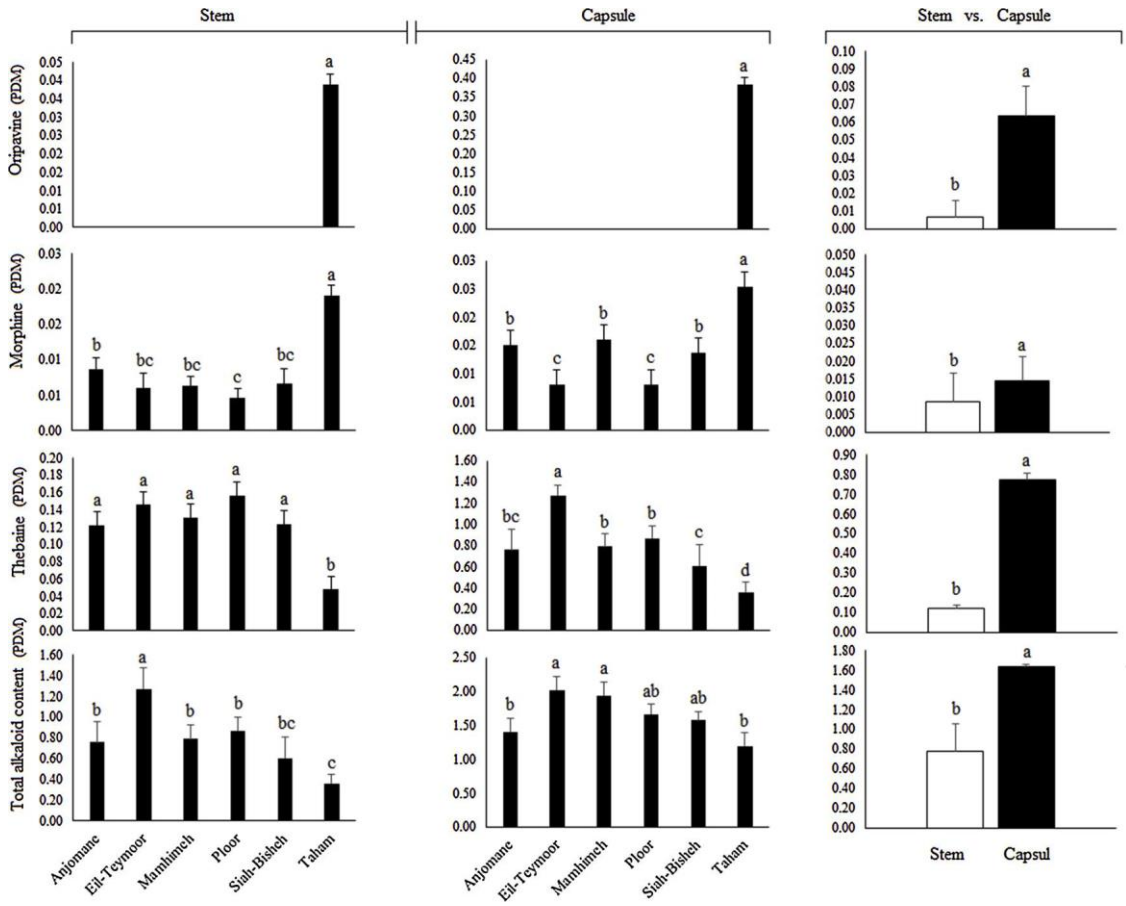

Fig. 4. Alkaloids detected in stem and capsules organs of the different Iranian poppy populations. Different letters in each graph indicate significant differences at $P < 0.05$. PDM: percent on dry matter.

useful for future efforts to use these results for genetic analyses of the Papaver species. In the present study, two marker techniques, ISSR and SCoT, were used to evaluate the genetic diversity in the 72 accessions of P. bractateum. Our data showed that there is a wide genetic diversity between the studied populations. Similarly, the high level of poly-morphism has been reported in studies of P. somniferum (Saunders et al., 2001; Parmaksiz and Ozcan, 2011) and P. bractateum (Mohseni et al., 2015). As a secondary result, the efficiencies of ISSR and SCoT as novel gene-based markers for characterising the genetic diversity of P. brac-tateum were compared. The percentage of polymorphism was 100% for both markers. In the cases of marker informativeness indices, SCoT markers indicated higher TAB, TPB, Rp and MI. However, the mean of PIC value for ISSR was found to be 0.42 vs. 0.31 for SCoT markers. Out of these indices, PIC, Rp and MI provide a benchmark that can help to determine the potential of markers in genetic analyses. Based on results, the average of PIC value for ISSR markers was greater than that for SCoT, suggesting the good capability of this system to render the level of polymorphism in our materials. In contrast, the average of values Rp and MI of SCoT markers were more than those of ISSR, which revealed the high ability of the SCoT in the evaluation of genetic diversity in P. bractateum. These results are in agreement with Rajesh et al. (2015); Etminan et al. (2016, 2018a) and Pour-Aboughadareh et al. (2017, 2018), who reported that SCoT markers were more useful for the dis-section of genetic diversity and structure than other molecular marker techniques.

Based on the results of AMOVA, the genetic diversity observed be-tween populations is equal to that found within them, which suggests that all accessions in each population have the same genetic back-ground. These results were confirmed by inter-population differentia-tion (G_{ST}) and gene flow (Nm) parameters. Accordingly, the G_{ST} value for ISSR, SCoT and combined data was 0.511, 0.542 and 0.533, re-spectively, indicating that there is a large proportion of genetic di-versity between populations. On the other hand, Wright (1951) de-monstrated that if $Nm < 1$, local populations tend to differentiate. As shown in Table 3, we found low values of Nm when we used ISSR, SCoT and combined data. Hence, this result can be explained by the size and degree of isolation and distributions of pollens between different po-pulations (Dumolin-Lapegue et al., 1997). Our results show the acces-sions from six different eco-geographical regions from the north (Ploor and Siah-Bisheh), northwest (Mamhimah and Eil-Teymoor), west (An-jomane) and central (Taham) of Iran are genetically different from each other, suggesting that the genetic diversity of these regions almost significantly differ from the average of all the studied regions. Out of six populations, the highest value of the genetic variation parameters (Na, Ne, I, He and PPL) was observed for Taham populations using each marker system. Furthermore, to achievement a general result we se-lected three populations Taham, Ploor and Eil-Teymoor as divergent populations through combined data. The higher genetic diversity in these populations might be attributed to the frequency of allelic var-iation of these germplasms being affected by different climatic condi-tions (Ni et al., 2018).

The present study indicated that the clustering of accessions based on ISSR and SCoT markers were similar. The correlation between two Jaccard dissimilarity coefficients obtained by the Mantel test (Mantel, 1967) resulted in a high correlation ($r = 0.61$) between ISSR and SCoT, revealing that these two molecular markers are similar to each other. The results of neighbour-joining cluster analysis and PCoA using mo-lecular data indicated the genetic relationships did not agree with the geographical distribution. For instance, according to ISSR analysis, Taham and Ploor populations (sampled from center (Zanjan province)

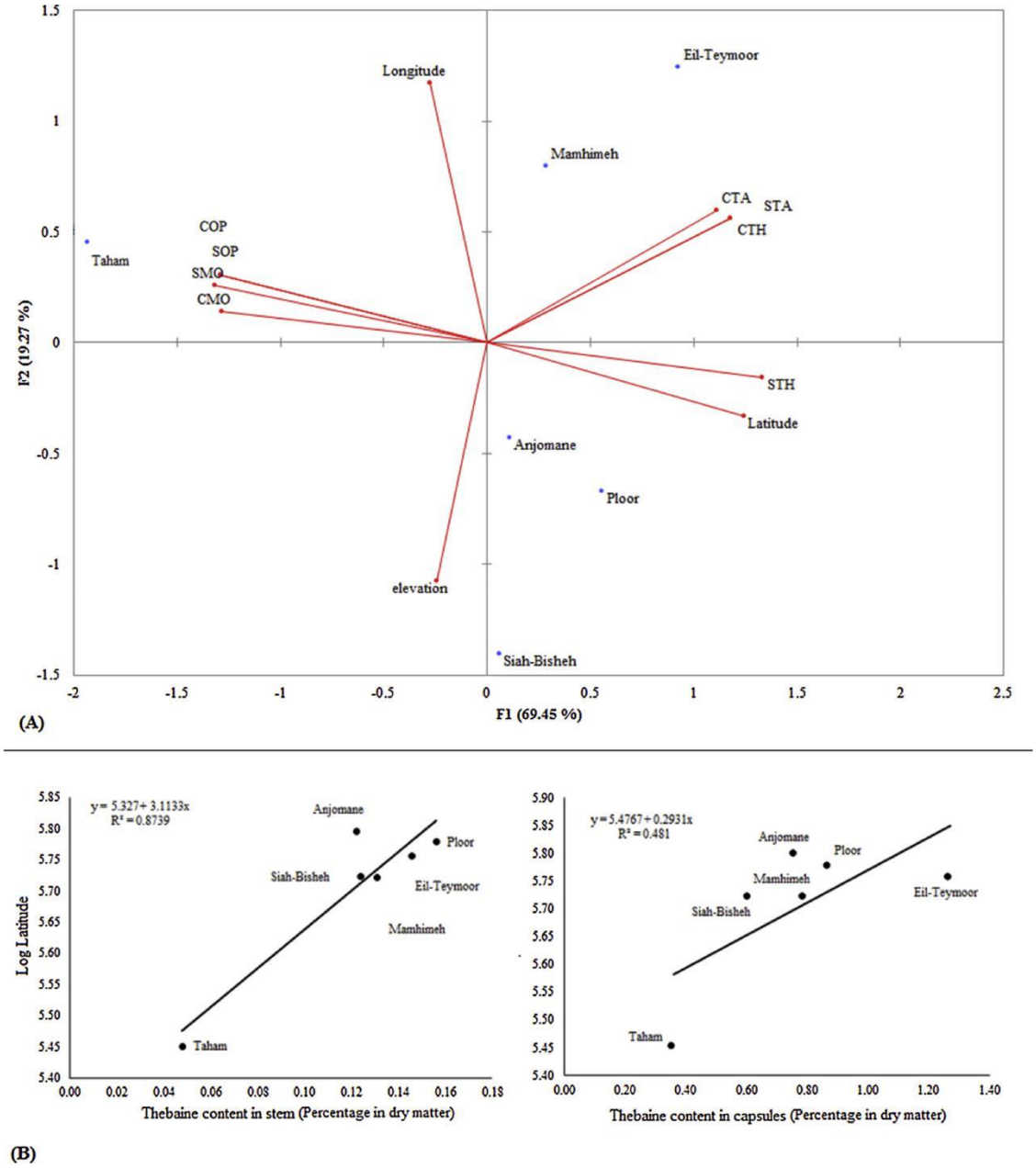

Fig. 5. Interrelationships among the different phytochemical traits and geographical parameters (A panel) and association among thebaine contents and latitude (B panel). COP, CMO, CTA, and CTH indicates oripavine, morphine, total alkaloids, and thebaine detected in plant capsules, respectively. SOP, SMO, STA, and STH indicates oripavine, morphine, total alkaloids, and thebaine detected in plant stem, respectively.

and north (Mazandaran province) regions of Iran, respectively) placed together in a same subpopulation, and on the contrary using SCoT analysis Mamhima (sampled form northwest of Iran (West Azarbaijan province)) and Taham (sampled from center of Iran (Zanjan province)) occur in the same sub-population. However, the combination of ISSR and SCoT revealed a clear clustering pattern which agrees with the geographical distribution of populations (Figs. 1 and 2). These results further are supported by Bayesian structure analysis (Fig. 3). In fact, this is the first report on population structure analysis on P. bractateum. Our results report 3 subpopulations—SP-1 including Ploor and Siah-Bisheh accessions, SP-2 including Taham accessions and SP-3 com-paring Eil-Teymoor, Anjomane and Mamhimah accessions—in Iranian poppy accessions. These results showed a high divergence between the studied populations and this is supported by AMOVA. In general, we can state that the use of a combination of ISSR and SCoT markers produce reliable results in genetic studies. Similarly, Alikhani et al. (2014) used a combination of ISSR, IRAP and SCoT to determination of population structure of Persian oak. Tiwari et al. (2016) estimated 6 and 4 subpopulations among 39 Kalmegh accessions using ISSR and SCoT markers, respectively. Etminan et al. (2018b) differentiated dif-ferent Salvia species into 3 subpopulations with a combination of ISSR and SCoT markers.

Although opium poppy (P. somniferum) is the global commercial source of medicinal opiates and related compounds, P. bracteatum has been identified as an alternative species with the potential to become a new opiate producing crop worldwide (Madam, 2011). The main medicinal components of the last species including codeine, narcotine, morphine and thebaine, which are widely used in the pharmaceutical industry as analgesics and anti-spasmodics (Schmeller and Wink, 1998). The oripavine and thebaine are thought to be the enol-methyl-ethers of morphinone and codeinone, respectively (Hosztafi, 2014). Thebaine is one of the ideal alkaloids that can be transformed into several opiates

(Chaudhary et al., 2009. There are several lines of evidence indicating that there is P. bracteatum which contains thebaine but not codeine and morphine (Hodges et al., 1977) may be considered to be a source of thebaine which cannot be altered in illicit drugs (Ataee et al., 2016). In the present study, the results of HPLC analysis showed the higher amount of total alkaloid, morphine, thebaine, oripavine in capsules than in stems (Fig. 4). Moreover, we found a wide range of variation among different populations for these alkaloids. As shown in Fig. 4, the oripaverine only was found in capsules of the Taham population, while the highest content of thebaine was found in the Eil-Teymoor followed by Ploor and Anjomane populations. Unlike Hodges et al. (1977), in this study, we found a considerably content of the alkaloid morphine in the Taham, Mamhimah and Anjomane populations. Hence, this finding supports the high potential for the Iranian poppy to be used as a good source of opiates in future industrial production. We also found that Eil-Teymoor followed by Mamhimah showed a greater amount of total alkaloids in their capsules than the other populations.

To study the interrelationships among different alkaloids and three geographical parameters, a principal component analysis was per-formed and its result showed that there was a strong correlation be-tween oripavine and morphine. Also, thebaine significantly correlated with the total alkaloid content (Fig. 5A). Interestingly, among the geographical parameter, latitude showed a significant and positive correlation with thebaine extracted from both stem and capsules and regression results confirmed these associations (Fig. 5B). Hence, the superior populations for thebaine contents including the Eil-Teymoor, Ploor and Anjomane populations. Also, our results reveal that the plant capsule has higher amounts of alkaloids, with thebaine being an abundant constituent.

5. Conclusion

Genetic diversity analysis serves as a key strategy in plant im-provement. This study provides a detailed understanding of the popu-lation structure and genetic diversity of the Iranian poppy germplasm. Our results revealed the high level of diversity between P. bracteatum populations and the different populations indicated their closeness to each other, confirming their association within the diverse gene pool. These results will support breeders in expanding the genetic back-ground of breeding accessions and utilizing the studied P. bracteatum resource more effectively. Moreover, it was found that three popula-tions Ploor, Eil-Teymoor and Anjomane due to their high contents of alkaloids like thebaine as well as Taham population due to its high content of morphine and oripavine have the potency for use in the pharmaceutical industry. Hence, the existence of wide genetic diversity in Iranian poppy accessions revealed by molecular markers and phy-tochemical traits in this study could help in the selection of appropriate populations for exploitation in varied uses and for further utilization in conservation programmes.

Author contribution

AQ, MO, AM, AE conceived and design the experiment. AM, AQ and JS carried out the experiment. AP and AQ performed the analysis. AP wrote the manuscript with significant inputs from PP. All authors have read and approved the final manuscript.

References

Addisonsoft XLSTAT, 2008. Data Analysis and Statistics With Microsoft Excel. Addisonsoft, Paris.

Alikhani, L., Rahmani, M.S., Shabanian, N., Badakhshan, H., Khadivi-Khub, A., 2014. Genetic variability and structure of Quercus brantii assessed by ISSR, IRAP and SCoT markers. Gene 552, 176–183. https://doi.org/10.1016/j.gene.2014.09.034.

Ataee, N., Fooladi, J., Namaei, M.H., Rezadoost, H., Mirzajani, F., 2016. Biocatalysts screen in g of Papaver bracteatum Flora for thebaine transformation to codeine and morphine. Biocatal. Agric. Biotechnol. 9, 127–133. https://doi.org/10.1016/j.bcab.2016.12.006.

Bhattacharyya, P., Kumaria, S., Kumar, S., Tandon, P., 2013. Start codon targeted (SCoT) marker reveals genetic diversity of Dendrobium nobile Lindl., an endangered medicinal orchid species. Gene 529, 21–26. https://doi.org/10.1016/j.gene.2013.07.096.

Carolan, J.C., Hook, I.L.I., Chase, M.W., Kadereit, J.W., Hodkinson, T.R., 2006. Phylogenetics of Papaver and related genera based on DNA sequences from IST nu-clear tibosomal DANN and plastid trnL intron and trnL-F intergenic spacers. Ann. Bot. 98, 141–155. https://doi.org/10.1093/aob/mcl079.

Chaudhary, V., Leisch, H., Moudra, A., Allen, B., De Luca, V., Cox, D.P., Hudlicky, T., 2009. Biotransformations of morphine alkaloids by fungi: N-demethylations, oxida-tions, and reductions. Collect Czechoslov Chem Commun. 74, 1179–1193. https://doi.org/10.1135/cccc2009025.

Collard, B.C.Y., Mackill, D.J., 2009. Start codon targeted (SCoT) polymorphism: a simple, novel DNA marker technique for generating gene-targeted markers in plants. Plant Mol. Biol. Rep. 27, 86–93. https://doi.org/10.1007/s11105-008-0060-5.

Craker, L.E., Simon, J.S., 1991. Herbs, Spices, and Medicinal Plants: Recent Advances in Botany, Horticulture, and Pharmacology Vol. II. The Haworth Press Inc., Binghamton NY.

Dittbrenner, A., Lohwasser, U., Mock, H.P., Börner, A., 2008. Molecular and phyto-chemical studies of Papaver somniferum in the context of infraspecific classification. Acta Hortic. 799, 81–88. https://doi.org/10.17660/ActaHortic.2008.799.9.

Doyle, J.J., Doyle, J.L., 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull. 19, 11–15.

Dumolin-Lapegue, S., Demesure, B., Fineschi, S., Le Corre, V., Petit, R.J., 1997. Phylogeographic structure of white oaks throughout the European continent. Genetics 146, 1475–1487.

Earl, D.A., Vonholdt, B.M., 2012. Structure Harvester: a website and program for visua-lizing STRUCTURE output and implementing the Evanno method. Conserv. Genet. Resour. 4, 359–361. https://doi.org/10.1007/s12686-011-9548-7.

Etminan, A., Pour-Aboughadareh, A., Mohammadi, R., Ahmadi-Rad, A., Noori, A., Mahdavian, Z., 2016. Applicability of start codon targeted (SCoT) and inter-simple sequence repeat (ISSR) markers for genetic diversity analysis in durum wheat gen-otypes. Biotechnol. Biotechnol. Equip. 30, 1075–1081. https://doi.org/10.1080/13102818.2016.1228478.

Etminan, A., Pour-Aboughadareh, A., Mohammadi, R., Noori, A., Ahmadi-Rad, A., 2018a. Applicability of CAAT box-derived polymorphism (CBDP) markers for analysis of genetic diversity in durum wheat. Cereal Res. Commun. 46, 1–9. https://doi.org/10.1556/0806.45.2017.054.

Etminan, A., Pour-Aboughadareh, A., Noori, A., Ahmadi-Rad, A., Shooshtari, L., Mahdavian, Z., Yousefiazar-Khanian, M., 2018b. Genetic relationships and diversity among wild Salvia accessions revealed by ISSR and SCoT markers. Biotechnol. Biotechnol. Equip. 32, 610–617. https://doi.org/10.1080/13102818.2018.1447397.

Feng, S., He, R., Yang, S., Chen, Z., Jiang, M., Lu, J., 2015. Start codon targeted (SCoT) and target region amplification polymorphism (TRAP) for evaluating the genetic relationship of Dendrobium species. Gene 567, 182–188. https://doi.org/10.1016/j. gene.2015.04.076.

Fiehn, O., 2002. Metabolomics the link between genotypes and phenotypes. Plant Mol. Biol. 48, 155–171. https://doi.org/10.1023/A:1013713905833.

Godwin, I.D., Aitken, E.A., Smith, L.W., 1997. Application of inter simple sequence repeat (ISSR) markers to plant genetics. Electrophoresis 18, 1524–1528. https://doi.org/10.1002/elps.1150180906.

Goldblatt, P., 1974. Biosystematic studies in Papaver section Oxytona. Ann. Mo. Bot. Gard. 61, 264–296.

Hagel, J.M., MacLeod, B.P., Facchini, P.J., 2007. Opium poppy. Biotechnol. Agric. For. 61, 169–187. https://doi.org/10.1007/978-3-540-71711-9_9.

Hodges, C.C., Horn, J.S., Rapoport, H., 1977. Morphinan alkaloids in Papaver bracteatum: biosynthesis and fate. Phytochemistry 16, 1939–1942. https://doi.org/10.1016/ 0031-9422(77)80100-3.

Hosztafi, S., 2014. Recent advances in the chemistry of oripavine and its derivatives. Adv. Biosci. Biotechnol. 5, 704–717. https://doi.org/10.4236/abb.2014.58084.

Jaccard, P., 1908. Nouvelles recherches sur la distribution florale. Bulletin de la Societe Vaudoise [New research on the floral distribution. Sci. Nat. 44, 223–270.

Kalia, R.K., Rai, M.K., Kalia, S., Singh, R., Dhawan, A.K., 2011. Microsatellite markers: an overview of the recent progress in plants. Euphytica 177, 309–334. https://doi.org/10.1007/s10681-010-0286-9.

Kyslikova, E., Babiak, P., Stepanek, V., Zahradnik, J., Palyzova, A., Maresova, H., Valesova, R., Hajicek, J., Kyslik, P., 2013. Biotransformation of codeine to 14-OHcodeine derivatives by Rhizobium radiobacter. J. Mol. Catal. 87, 1–5. https://doi.org/10.1016/j.molcatb.2012.10.004.

Li, J.M., Jin, Z.X., 2008. Genetic structure of endangered Emmenopterys henryi Oliv. Based on ISSR polymorphism and implications for its conservation. Genetica 133, 227–234. https://doi.org/10.1007/s10709-007-9204-z.

Madam, P.R.K., 2011. Aspects of the Biology of Papaver bracteatum Lind, a New Crop for Tasmania, PhD Thesis. University of Tasmania.

Mantel, N., 1967. The detection of disease clustering and a generalized regression ap-proach. Cancer Res. 27, 209–220.

Milo, J., levy, A., Palevitch, D., 1998. The cultivation and breeding of P. bracteatum. In: Bernath, J. (Ed.), Poppy - The Genus Papaver. Harwood Academic Publisher, Amesterdam, pp. 279–289.

Mohseni, Z., Bernousi, I., Mandoulakani, B.A., Darvishzadeh, R., 2015. Genetic diversity in Papaver bracteatum and Papaver somniferum populations revealed by ISSR markers. Bulg. J. Agric. Sci. 21, 485–493.

Moradkhani, H., Mehrabi, A.A., Etminan, A., Pour-Aboughadareh, A., 2015. Molecular diversity and phylogeny of Triticum-Aegilops species possessing D genome revealed by SSR and ISSR markers. Plant Breed Seed Sci. 71, 81–95. https://doi.org/10.1515/ plass-2015-0024.

Nakagawa, A., Minami, H., Kim, J.S., Koyanagi, T., Katayama, T., Sato, F., Kumagai, H., 2011. A bacterial platform for fermentative production of plant alkaloids. Nat. Commun. 2, 326. https://doi.org/10.1038/ncomms1866.

Newman, D.J., Cragg, G.M., 2012. Natural products as sources of new drugs over the 30 years from 1981 to 2010. J. Nat. Prod. 75, 311–335. https://doi.org/10.1021/ np200906s.

Ni, J.L., Zhu, A.G., Wang, X.F., Xu, Y., Sun, Z.M., Chen, J.H., Luan, M.B., 2018. Genetic diversity and population structure of ramie (Boehmeria nivea L.). Ind. Crops Prod. 115, 340–347. https://doi.org/10.1016/j.indcrop.2018.01.038.

Niknam, S., Faramarzi, M.A., Abdi, K., Yazdi, M.T., Amini, M., Rastegar, H., 2010. Bioconversion of codeine to semi-synthetic opiate derivatives by the cyanobacterium Nostoc muscorum. World J. Microbiol. Biotechnol. 26, 119–123. https://doi.org/10.1007/s11274-009-0150-z.

Nyman, U., 1978. Selection for high thebaine/low morphine content (cpv. Morph: the) in Papaver somniferum L. Hereditas 89, 43–50. https://doi.org/10.1111/j.1601-5223.1978.tb00979.x.

Parmaksiz, I., Ozcan, S., 2011. Morphological, chemical, and molecular analyses of Turkish papaver accessions (Sect. Oxytona). Turk. J. Bot. 35, 1–16. https://doi.org/ 10.3906/bot-1003-39.

Peakall, R., Smouse, P.E., 2006. GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. Mol. Ecol. Notes 6, 288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x.

Perrier, X., Flori, A., Bonnot, F., 2003. Data analysis methods. In: Hamon, P., Seguin, M., Perrier, X., Glaszmann, J.C. (Eds.), Genetic Diversity of Cultivated Tropical Plants, pp. 43–76 London, Plymouth, UK.

Poczai, P., Varga, I., Laos, M., Cseh, A., Bell, N., Valkonen, J.P., 2013. Advances in plant gene-targeted and functional markers: a review. Plant Methods 9, 1–31. https://doi.org/10.1186/1746-4811-9-6.

Pour-Aboughadareh, A., Ahmadi, J., Mehrabi, A.A., Etminan, A., Moghaddam, M., 2017. Assessment of genetic diversity among Iranian Triticum germplasm using agro-morphological traits and start codon targeted (SCoT) markers. Cereal Res. Commun. 45, 574–586. https://doi.org/10.1556/0806.45.2017.033.

Pour-Aboughadareh, A., Ahmadi, J., Mehrabi, A.A., Etminan, A., Moghaddam, M., 2018. Insight into the genetic variability analysis and relationships among some Aegilops and Triticum species, as genome progenitors of bread wheat, using SCoT markers. Plant Biosyst. 152, 694–703. https://doi.org/10.1080/11263504.2017.1320311.

Pritchard, J.K., Stephens, M., Donnelly, P., 2000. Inference of population structure using multilocus genotype data. Genetics 155, 945–959.

Rajesh, M.K., Sabana, A.A., Rachana, K.E., Rahman, S., Jerard, B.A., Karun, A., 2015. Genetic relationship and diversity among coconut (Cocos nucifera L.) accessions revealed through SCoT Analysis. 3 Bitech. 5, 999–1006. https://doi.org/10.1007/ s13205-015-0304-7.

Saunders, J.A., Pedroni, M.J., Penrose, D.J., Fist, A.J., 2001. AFLP analysis of opium poppy. Crop Sci. 41, 1596–1606. https://doi.org/10.2135/cropsci2001.4151596x.

Schmeller, T., Wink, M., 1998. Utilization of alkaloids in modern medicine. In: Roberts, M.F., Wink, M. (Eds.), Alkaloids- Biochemistry, ecoLogy and Medicinal Applications. Plenum Press, New York, pp. 435–459.

Sharghi, N., Lalezari, I., 1967. Papaver bracteatum Lindl., a highly rich source of the-baine. Nature 213, 1244. https://doi.org/10.1038/2131244a0.

Tiwari, G., Singh, R., Singh, N., Choudhury, D.R., Paliwal, R., Kumar, A., Gupta, V., 2016. Study of arbitrarily amplified (RAPD and ISSR) and gene targeted (SCoT and CBDP) markers for genetic diversity and population structure in Kalmegh [Andrographis paniculata (Burm.f.) Nees]. Ind. Crops Prod. 86, 1–11. https://doi.org/10.1016/j.indcrop.2016.03.031.

Vos, P., Hogers, R., Bleeker, M., Reijans, M., Van de Lee, T., Hornes, M., Freijters, A., Pot, J., Pelemen, J., Kuiper, M., Zabeau, M., 1995. AFLP: a new concept for DNA fin-gerprinting. Nucleic Acids Res. 21, 4407–4414. https://doi.org/10.1093/nar/23.21. 4407.

Wang, C., Zhang, H., Qian, Z.Q., Zhao, G.F., 2008. Genetic differentiation in endangered Gynostemma pentaphyllum (Thunb.) Makino based on ISSR polymorphism and its implications for conservation. Biochem. Syst. Ecol. 36, 699–705. https://doi.org/10.1016/j.bse.2008.07.004.

Williams, J.G.K., Kubelik, A.R., Livak, K.J., Rafalski, J.A., Tingey, S.V., 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res. 18, 6531–6535. https://doi.org/10.1093/nar/18.22.6531.

Wolfe, A.D., Liston, A., 1998. Contribution of PCR based methods to plant systematic and evolutionary biology. In: Soltis, P.S., Soltis, D.E., Doyle, J.J. (Eds.), Molecular Systematic of Plants II. Chapman and Hall, New York, pp. 43–86.

Wright, S., 1951. The genetical structure of populations. Ann. Eugen. 15, 323–354. https://doi.org/10.1111/j.1469-1809.1949.tb02451.x.

Yeh, F.C., Yang, R.C., Boyle, T., Ye, Z.H., Mao, J.X., 1997. POPGENE, the User-Friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Center, University of Alberta, Edmonton.

Zietkiewicz, E., Rafalski, A., Labuda, D., 1994. Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. Genomics 20, 176–183. https://doi.org/10.1006/geno.1994.1151.